Combinatorial Biosynthesis of Tetrahydrocannabinol

The LEGO-Principle to construct an artifical biosynthetic pathway for THCA Muntendam, R, Czepnik, M, Schütz, V, Arndt, T, Quentmeier, A, and Kayser, O

SCIENTIFIC HIGHLIGHTS

Plants and microorganisms are important sources for gene and drug discovery. Most isolated natural products with drug potential are structurally too complex, their isolation is too expensive or they can only be obtained in very low quantities. To overcome these problems, for combinatorial biosynthesis plant pathways are copied in microbial organisms acting as biological factories to produce natural compounds of interest.

Tetrahydrocannabinol (THC) (7) is a natural product from the plant *Cannabis sativa* (Fig.1). Besides of the problematic legal situation the compound has as well a high medical potential. It is used as antiemetic drug in patients suffering from cancer and as pain relief for Multiple Sclerosis patients.



Fig. 1: Cannabis sativa, cultivated in Buitenpost, NL

Today the isolation from plants is legally problematic or too expensive by organic synthesis. Biotechnological production in genetically modified baker yeast is a solution to these problems. We assemble the plant pathway to express heterologous genes and to analyze host metabolom for optimal production yield. In addition we clone human liver cytochromes into microbiological hosts to produce human metabolites for forensic chemistry.

At all major biocatalytic steps in the biosynthesis (Fig. 2) we study the catalytic activity and optimize enzyme activity by site directed mutagenesis.

<u>Highlight:</u> Identification and sequencing of the CBGA synthase, an important enzyme in the biosynthesis of CBGA (5) by prenylation of olivetolic acid (4). The mode of action is studied to suggest a 3d structure by protein modeling and to allow enzyme mutations.

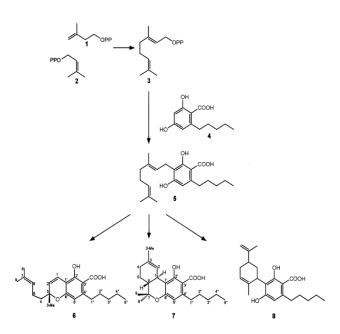


Fig. 2: Biosynthesis of THCA (7)

<u>Highlight:</u> Verifying the protein structure of THCA-Synthase. This enzyme catalysis the ring forming step from CBGA (5) to THCA (7). By site directed mutagenesis the mode of catalysis is studied and new mutants produced to confirm the calculated model. In addition THCA-Synthase is codon optimized for improve expression and acitivity in *S. cerevisiae* as potential host.

<u>Highlight</u>: Assembly of the biosynthetic genes to produce THC in *E. coli* under feeding conditions with biosynthetic precursors. The biosynthesis was successfully carried out to produce recombinant THCA.

<u>Highlight:</u> Recombinant synthesis of human THC metabolites. Human liver enzymes (Cyp450) are cloned and expressed in *E. coli.* Incubation with THC led to the production of human metabolites which can be used as references compounds in forensic chemistry.

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<u>Key Words</u>: Cannabis sativa, THC, CBGA, prenyl transferases, cannabinoids, metabolic engineering, biocatalysis, cytochrome

Publications:

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